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## **Potentially Actionable Suspect Samples and Cyst Nematodes Affecting Potatoes**

A Potentially Actionable Suspect Sample (PASS) is, in its simplest form, a sample of a pest or pathogen that has been presumptively identified as an agent of regulatory concern. This means that Federal action would result from the confirmation and/or that Federal funds would be utilized in response to the confirmation of the pest or pathogen. Under these circumstances, confirmatory testing by a Federal laboratory would be required.

Generically, a PASS sample is a presumptive identification of a pest or pathogen of Federal regulatory concern, as per the Plant Protection Act (2000). This is typically a specimen(s) that was identified by an external laboratory or screening facility. The specimen itself or its DNA is then forwarded to a designated Federal laboratory for confirmatory testing. Subsequent samples from a pre-defined area around the first sample would not require confirmatory testing, but new finds outside of the area would be treated as new PASS samples. Typically, PASS samples would also encompass any sample that involves unusual or unexpected circumstances, such as a new host, new location, etc. This policy is specific to any pest or pathogen and based on what is known on the biology of the organism and epidemiology of the disease.

### **‘PASS’ POLICY FOR PALE CYST NEMATODE (*Globodera pallida*) AND GOLDEN NEMATODE (*Globodera rostochiensis*)**

Complete, definitive identification of *G. pallida* or *G. rostochiensis* is a multi-step process:

- 1) reading/sorting of soil extracts and removing anything resembling a cyst,
- 2) examination, whereby structures resembling cysts are examined by individuals with training such that they can conclude a reasonable probability that the removed bodies are nematode cysts,
- 3) verification that the sample contains *Globodera* spp or other cyst nematode genera (such as *Cactodera*),
- 4) verification that the suspect nematode cysts and/or any juvenile forms have key characters and are morphometrically within the range of the target species,
- 5) verification that the suspect nematode tissue yields DNA identifiable as the suspect species,
- 6) when morphological and PCR results do not agree with each other, sequencing the DNA may be necessary and additional samples may need to be analyzed before reporting of results by NIS and,
- 7) at least two cysts from two different samples with one of those cysts containing live eggs/juveniles would be required before a declaration of infestation is made. If only one sample with cysts is found that may contain live juveniles, additional surveys of the area are required to confirm the find.

The overriding principle is that if there is any doubt about the identity of a sample, it may either require additional sampling of the area, further testing or both.

Federal confirmatory testing in the case of a presumptive positive (= suspect sample) includes identification on the basis of cyst morphology and testing of DNA isolated from J2 juveniles from eggs from one or more cysts. Morphometric testing is done at the ARS Nematology Laboratory. DNA testing is done by APHIS PPQ laboratories, but may also be performed by the ARS Laboratory if needed to corroborate results.

**A. Regulated Areas ('Suspect' samples from within a regulated area):**

A potentially positive or 'suspect' sample from known to be infested field is not a PASS sample. This means that these samples do not require full Federal confirmatory testing. Positive identification of *G. pallida* or *G. rostochiensis* on the basis of morphology will be sufficient. However, a non-infested field within a regulated area will require full Federal confirmatory testing including DNA analysis.

**B. Non-Regulated Areas ('Suspect' samples from within a non-regulated area):**

A potentially positive or 'suspect' sample from any field outside the regulated area IS a PASS sample. That means that these samples must receive full Federal confirmatory testing. Positive identification of *G. pallida* or *G. rostochiensis* will be on the basis of morphology **AND** DNA analysis.